A MOTILE STRAIN OF *FUSOBACTERIUM POLYMORPHUM*

HEINER HOFFMAN

Department of Bacteriology, The Ohio State University, Columbus 10[1]

The taxonomic system currently used by American bacteriologists (Breed et al., 1948) recognizes four species of the genus *Fusobacterium*. Three of these were originally described by Knorr (1923), while the fourth was described by Weinberg and Prévot (1926) three years later. None of these strains are in the American Type Culture Collection (ATCC), and indeed when our studies on the fusobacteria began, the ATCC had no cultures of this genus at all. Of the several strains we isolated and studied some years ago, only one was thoroughly characterized, and it was deposited with the ATCC in 1951 as *Fusobacterium polymorphum* (ATCC No. 19053) although it differs significantly in certain respects from the original description of the species by Knorr.

In recently reviewing the literature for our revision of the genus *Fusobacterium* in the forthcoming seventh edition of "Bergey's manual of determinative bacteriology", it became apparent that certain of our observations with the strain we characterized as *F. polymorphum* were still unique. We therefore wish to record our observations as documentation for a revision of the species, and to provide a description for the culture deposited with the ATCC.

OBSERVATIONS

The microorganism was isolated from the labial gingival crevice area of the maxillary central incisors of an adolescent male. The gingiva was hyperemic and hypertrophic, apparently largely from poor oral hygiene, but no breakdown of the periodontal membrane was observed. For initial cultivation the inoculum was streaked out upon a series of Difco beef brain heart infusion blood agar plates and incubated 3 days in a screw top jar utilizing the Buchner pyrogallol technique for anaerobiosis. A typical isolated colony was picked upon examination with the dissecting microscope and subcultured into thioglycollate broth (Baltimore Biological Laboratory) enriched with ascitic fluid.

After unsuccessful attempts to continue surface plate cultivation it was necessary to abandon the pyogallol anaerobic technique for the Brewer modification of the Brown jar. Subsequent studies (Hoffman, 1951) established that this strain, as well as the other seven studied, has an absolute carbon dioxide requirement which may perhaps not be satisfied by the conventional pyrogallol technique. However, it appeared that the most serious difficulty with Buchner's method is the evolution of carbon monoxide upon oxidation of the pyrogallol. Attempts failed to obtain surface growth on blood agar plates incubated aerobically, but it was found that a small amount of residual oxygen in the Brewer jar did not hamper optimal growth.

Following solution of the problem of gaseous needs for cultivation, a number of conventional media and a few special media were tested for their ability to support growth. Maximum growth was obtained from several of the more complex media, and especially from those with beef tissue infusion base supplemented with rabbit blood or serum. The potato extract medium of Slanetz and Rettger (1933) was excellent but proved far too time-consuming in its preparation for routine use.

Colonial morphology of the present strain on blood agar during the first three months of subculture consisted predominantly of large, heaped-up, cream colored growth with a somewhat mulberry-like surface. Occasional rhizoid colonies also

[1]Present address: Department of Microbiology, College of Dentistry, New York University, New York, N. Y.

appeared. The colony form then changed completely to a type which had been appearing regularly almost from the first. This was a smooth-edged, slightly bluish-tinged brilliant white colony, convex and quite regular, which was punctiform after 24 hr., about 1 mm. in diameter after 48 hr. (fig. 1) and 1½ to 3 mm. in diameter after 72 hr. Upon close inspection it was seen that the growth presented a peculiar mottled effect of white flecks in a water-clear matrix. With continued incubation this type of colony transformed into a "rough" stage as a result of flat peripheral growth from the colony's edge (fig. 2). The colony in this late phase was 3 to 6 mm. in diameter, and its color was markedly different from early growth. The original convex primary growth changed to light golden yellow, while the secondary peripheral zone was gray. Some time later it was noted that the young colonies no longer presented the brilliant white color, but rather were a comparatively dull gray. The consistency of the growth is butyrous; a slight greening hemolysis develops within 24 hr. of continuous exposure of the plates to air. In early cultivations good growth was accompanied by a strongly putrid odor, but this characteristic tended to disappear with continued transfers.

The gross appearance of broth cultures depends upon the medium used. With Slanetz and Rettger's-potato extract broth, a heavy turbidity initially appears which goes on to form a grayish sediment that settles again quickly following agitation. Microscopic examination of cells from potato extract broth revealed regularly formed single or double rods with characteristic sharply—pointed fusiform shape. The cells in enriched thioglycollate broth (fig. 3) appeared almost as regular and characteristic in morphology as in the potato extract medium. Not infrequently, however, numerous globular extrusions and misshapen cells (fig. 4) were found in the enriched thioglycollate, especially in the earlier cultivations, which appeared similar to the "large bodies" observed by Dienes and Smith (1944) in *Bacteroides*. Long filaments often appeared upon solid media, or in broth cultures subjected to suboptimal conditions. Thus in potato extract broth at pH 10.4 there were formed braids and tangles of long filaments (fig. 5), while in the same broth at pH 5.1 the growth was also filamentous but distinguished by numerous fusiform swellings and by failure to braid (fig. 6).

However, cells which appeared to have developed optimally in broth were of uniform size. Upon measurement with a calibrated ocular micrometer it was found that the length ranged from 4 to 13 μ, but most of the cells clustered around 8 to 13 μ. The width was slightly short of 0.5 μ.

The Gram staining reaction (Kopeloff and Beerman technique) was always negative although prominent intracellular granules were present which sometimes gave a positive reaction. Later in cultivation when the granules also gave a consistent negative reaction, they nevertheless counterstained more deeply with the safranine than did the rest of the cell. An intensive cytochemical study of these granules (Hoffman, 1951) later revealed they contain both pentose and desoxypentose nucleic acids.

Wet mounts and hanging drop preparations of enriched thioglycollate broth cultures revealed a slow motion of translation and a distinctive vibratory movement

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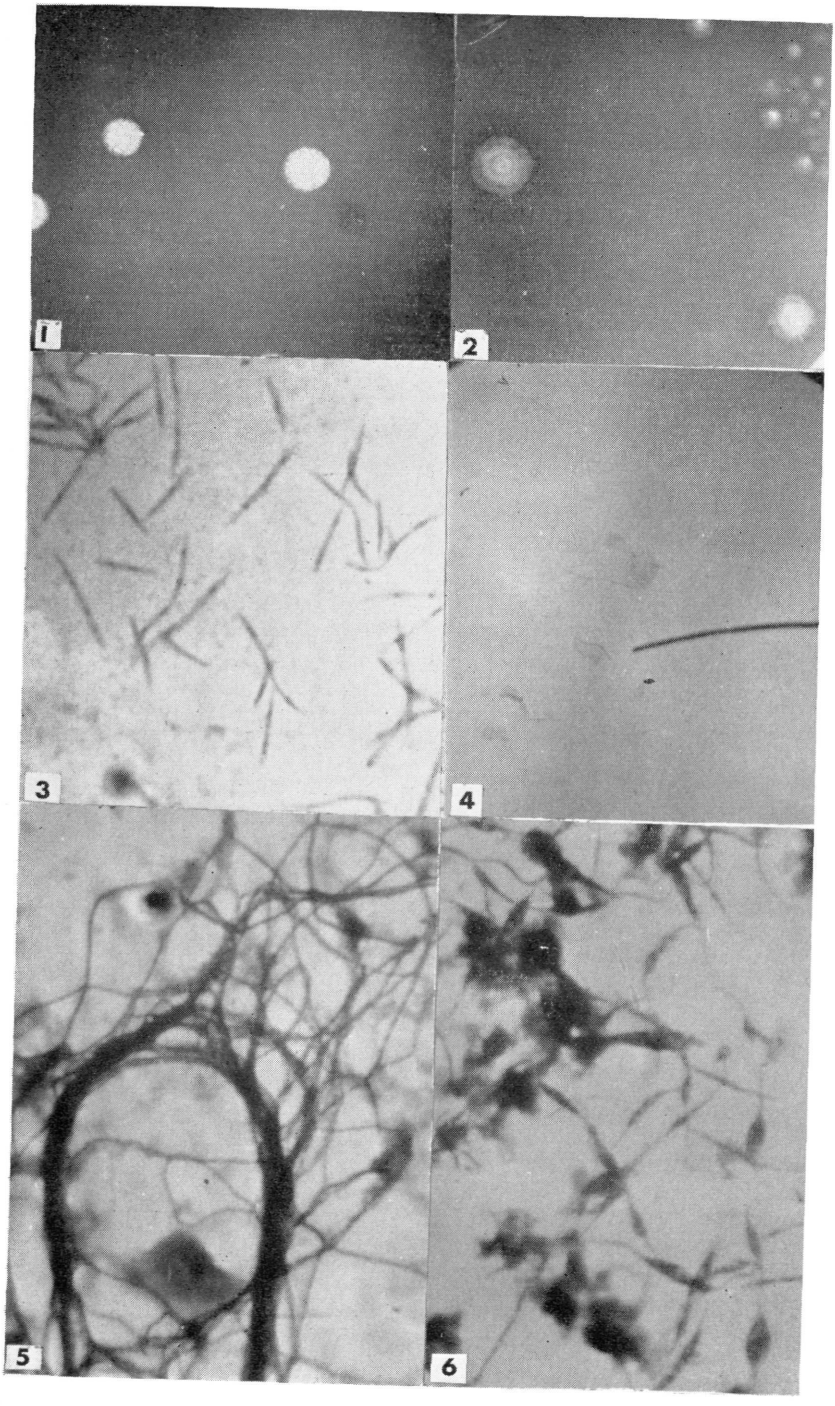
EXPLANATION OF FIGURES IN PLATE

1. Two day old colonies on rabbit blood beef brain-heart infusion agar.
2. Aged (five day) colonies on blood agar.
3. Three day thioglycollate—ascites culture, methylene blue stain (2000X).
4. Three day thioglycollate culture, "large body" formation, Gram stain (1600X).
5. Filament and braid formation at pH 10.4 in potato extract broth, 38 hour culture, methylene blue stain (1600X).
6. Filament and fusiform swelling formation at pH 5.1 in potato extract broth, 38 hour culture, methylene blue stain (1600X).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of the bacillary ends. Nevertheless, persistent attempts to demonstrate flagella by the conventional staining methods revealed only an elaborated arborized amorphous mass surrounding the entire cell. No evidence for typical bacterial flagella was found.

Of the eight carbohydrates tested (table 1) in potato extract broth, only dextrose, fructose and galactose were fermented. The fermented tubes only showed a drop to pH 6.4 compared to the control pH 6. 9 after 7 days incubation. No gas appeared in the Dunham fermentation tubes. The Barritt alpha-naphthol test for acetylmethylcarbinol was negative after 3, 5 and 6 day readings; a weak evolution

TABLE 1

Characteristics of the present strain compared to the fusobacterial species described in Bergey's manual of determinative bacteriology (Breed, et al., 1948)

Characteristic	*Present strain*	*F. polymorphum*	*F. plauti-vincenti*	*F. biacutum*	*F. nucleatum*
Size	less than 0.5 μ by 8 to 13 μ, long filaments common	0.2 to 0.5 μ by 8 to 16 μ, long filaments common	0.5 to 1.0 μ by 8 to 16 μ (double cells), sometimes short chains or long filaments	0.4 to 0.5 μ by 1.4 to 3.0 μ, sometimes in short chains	1.0 by 4.0 μ spindle shape is marked
Gram reaction	negative	negative	negative	negative	negative
Motility	vibratory and translation	negative	negative	negative	negative
Temperature optimum	37° C	35° C to 37° C	35° C to 37° C	not reported	35° C to 37° C
pH optimum	6.9 to 7.7	7.0 to 8.2	6.8 to 8.0	not reported	6.8 to 8.2
Odor	strongly putrid in early culture	negative	negative	not reported	positive
H^2S	weakly positive	negative	negative	positive	negative
Gas	negative	negative	negative	not reported	negative
Indole	positive	conflicting reports	negative	negative	negative
Gelatin	liquefied	not liquefied	not reported	not liquefied	not liquefied
Carbohydrate fermentations:					
dextrose	acid	acid usually	acid	acid	acid
fructose	acid	acid usually	acid	acid	acid
galactose	acid	not reported	not reported	acid	not reported
inulin	negative	negative	negative	not reported	negative
lactose	negative	negative	acid variable	acid	acid variable
maltose	negative	negative	acid	acid, gas	negative
mannitol	negative	negative	negative	not reported	negative
sucrose	negative	acid usually	acid	not reported	acid variable

of hydrogen sulfide was demonstrated with lead acetate paper suspended in a thioglycollate broth culture for 3 days. Ascites-enriched gelatin was liquefied; the Ehrlich-Bohme test for indole was positive with 48 hr. culture in beef brain-heart infusion broth containing 0.1 percent tryptone. Heated blood slant cultures were catalase negative. Hydrolysis was very slight of 1 percent potato starch in beef brain-heart infusion agar.

The pH growth range was found to be 5.1 to 10.4 with optimum growth between 6.9 and 7.7. The temperature growth range was found to be 31° to 43° C, with the optimum at 37° C.

Cultures in enriched thioglycollate broth remained viable 6 to 7 days at 37° C, 20 days at room temperature and 3 to 4 mo. in the refrigerator at 4° C. Heated blood agar slant cultures were viable for at least 8½ mo., but not 10 mo. at—45° C,

while such tubes with the oxygen absorbed by pyrogallol (Wright tubes) gave viable cultures for at least 22 mo. Lyophilized cultures were viable after 5½ yr., when the last test was made.

It was found that 3-day culture in ascites enriched thioglycollate broth incubated at 37° C survived 24 hr. exposure to oxygen, but not 41 hr. The same culture resisted 52° C for 15 min., 56° C for 10 min., 58° C for 5 min. and 60° C for 2 min.

Neither fibrinolysin nor hyaluronidase could be demonstrated. Pathogenicity could not be demonstrated in white mice with 48 hr. cell suspension from blood agar cultures upon intraperitoneal, intravenous or subcutaneous inoculation. Guinea pig inoculations by the intraperitoneal, subcutaneous and intradermal routes also were negative. Both white mice and guinea pigs were observed for three weeks following inoculation.

DISCUSSION

The characteristics of the microorganism studied here clearly establish it as belonging to the genus *Fusobacterium*, but its species identification is not as clear. Morphology and carbohydrate fermentations resemble those of *F. polymorphum* (see table 1), but its ability to produce hydrogen sulfide and indole, its liquefaction of gelatin, and the evolution of a strongly putrid odor in early culture all indicate a proteolytic activity more pronounced than that of any of the species described in Bergey's manual.

The most distinctive difference in the present strain from the species recognized by the American taxonomists is the peculiar vibratory motion which apparently has not been observed in any other group of bacteria.

The changes which were noted in colonial morphology are extremely interesting. They would seem to indicate an adjustment to artificial media which involved profound changes in the biological characteristics of the strain. We were unable to maintain cultures giving surface colonies characteristic of the early cultivations, and obtained no clues to the factors which may have influenced the transition to the colonial form obtained now.

The marked cellular morphological plasticity we observed seemed to be largely expressed as a reaction to deviations from optimal cultural conditions. When the medium for cultivation was optimum, we consistently obtained cells which corresponded to the description for *F. polymorphum*. This, and a pattern of carbohydrate fermentations consistent with *F. polymorphum*, is sufficient evidence in our opinion to warrant identification of the present strain as *F. polymorphum*. It must be remembered in this regard that Knorr's original description of the species was based upon techniques which are now known to be inadequate; moreover, the original strain apparently has been lost and is no longer available for study. Therefore, rather than increase the number of fusobacterial species, it seems more satisfactory simply to amend the classical description of *F. polymorphum*.

SUMMARY

A motile strain of fusiform bacillus is described. Its cultural characteristics indicate it is *Fusobacterium polymorphum* although it differs significantly from the classical description by possessing a peculiar vibratory motion as well as having movement of translation.

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REFERENCES

Breed, R. S., E. G. D. Murray, and **A. P. Hitchens.** 1948. Bergey's manual of determinative bacteriology. Ed. 6. Baltimore. Williams and Wilkins Company, p. 581–583.

Dienes. L., and **W. E. Smith.** 1944. The significance of pleomorphism in *Bacteroides* strains. Jour. Bact., 48: 125–153.

Hoffman, H. 1951. Gaseous requirements for the cultivation of fusobacteria. Jour. Bact. 61: 241–242.

———. 1951. The cytochemistry of bacterial nuclear structures. Jour. Bact. 62: 561–570.

Knorr, M. 1923. Ueber die fusospirilläre Symbiose, die Gattung *Fusobacterium* (K. B. Lehmann) and *Spirillum sputigenum*. II. Mitteilung. Die Gattung *Fusobacterium*. Centralbl. f. Bakt. (Abt. 1, Orig.) 89: 4–22.

Slanetz, L. W., and **L. F. Rettger.** 1933. A systematic study of the fusiform bacteria. Jour Bact. 26: 599–621.

Weinberg, M., and **A. - R. Prévot.** 1926. Recherches sur la flore microbienne de l' appendicite *Fusobacterium biacutum* (n. sp.). Comp. rend Soc. de Biol. 95–522.